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Liposomal Lactoferrin as Potential Preventative and Cure for COVID-19

Gabriel Serrano[1,*], Iulia Kochergina[1], Arturo Albors[2], Eva Diaz[3], Mar Oroval[3], Guillen Hueso[3], Juan M Serrano[3]

[1]Dr. Serrano Dermatology Clinic, Valencia, Spain
[2]Medical Department of Sesderma Laboratories, Valencia, Spain
[3]Research Department of Sesderma Laboratories, Valencia, Spain

Corresponding author:
Dr. Gabriel Serrano
Dr. Serrano Dermatology Clinic, Valencia, Spain.
E-mail: gabriels@sesderma.com

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ABSTRACT

A prospective observational study was performed in 75 patients with typical symptoms of COVID-19 who tested positive to IgM/IgG rapid test. Patients were isolated and treated at home using remote systems, reviewed twice a day for 10 days, and followed up to 1 month. A liposomal bovine lactoferrin (LLF) nutritional syrup food supplement (32 mg of LF/10 ml plus 12 mg of vitamin C) was administered orally in 4 to 6 doses per day for 10 days. In addition, a zinc solution was administered at the dose of 10 mg/10 ml twice or three times a day. A control group of 12 patients who took only LLF was included. All family members in contact with patients (256 persons) were also treated with half of this dose. The treatment allowed a complete and fast recovery in all patients (100%) within the first 4-5 days. Same treatment at lower dose prevented the disease in healthy persons directly related with the affected patients. Weakness (95%) followed by a dysfunction in the perception of smelling and tasting (88%), cough (83%), muscular pain (67%) were the most frequent complains. LF possess antiviral, immunomodulatory and anti-inflammatory effects which might be important for the treatment of COVID-19 infection. We conclude that LLF potentially prevent and cure COVID 19 infection.

INTRODUCTION

In December 2019, coronavirus disease 2019 (COVID-19) emerged in Wuhan City and rapidly spread throughout China and to all five continents within a few months, creating a pandemic. Since then, search has been carried out for a safe and effective treatment. Clinically, the illness starts with fever, headache, dry cough, fatigue, myalgia, dyspnea, abdominal pain, diarrhea, nausea and vomiting. Disease progression may gradually lead to respiratory failure due to alveolar damage and even death.[1-3] Laboratory predictors[4] of adverse clinical outcomes include lymphopenia (35-75% of cases), increased values of CRP (75-93% of cases), LDH (27-92% of cases), ESR (up to 85% of cases) and D-dimer (36-43% of cases), as well as low concentrations of serum albumin (50-98% of cases) and hemoglobin (41-50%). Many laboratory abnormalities were instead predictive of adverse outcome, including increased white blood cell count, increased neutrophil count, decreased lymphocyte count, decreased albumin, increased LDH, ALT, AST, bilirubin, creatinine, cardiac troponins, D-dimer, prothrombin time, procalcitonin and CRP values.[4] Antivirals and hydroxychloroquine were initially suggested as therapies for COVID-19 associated pneumonia in multicenter clinical trials conducted in China.[5,6] Angiotensin Converting enzyme 2 (ACE2) is the main receptor for COVID-19 (Figure 1) and plays a vital role in the entry of the virus into the cell to induce lung infection.[7-15] ACE2 is highly expressed in the nose, mouth, epithelial respiratory tract, alveolar epithelial cells of the lungs, enterocytes of the small intestine and the brush border of the proximal tubules of the kidney.[10] The localization of ACE2 receptors is associated with the tissue tropism and pathogenesis of the viral infection. The disease may cause an upper respiratory tract (sinuses, nose, and throat) infection but most frequently a lower respiratory tract infection (trachea and lungs). The COVID-19 infection spreads the same way than other coronaviruses do, mainly through person-to-person contact. Infections range from mild to serious. In the year 2011, Lang *et al.* stated that Lactoferrin, could be potentially useful for the treatment of SARS disease.[7] However, until now, there have been no reports of the clinical use of LF in patients affected by SARS-COv or COVID-19 infection. The COVID 19 is an enveloped, RNA virus with a genome of about 30000 nucleotides in length and encodes a nonstructural replicase complex and structural proteins including, including spike (S), envelope (E), and S2, membrane (M) and nucleocapsid (N) proteins.[7] The spike protein is composed of two units: S1 which mediates the virus binding to receptors on target cells, and S2, which triggers virus and host cell membrane fusion.[7] Angiotensin-converting enzyme 2 (ACE2), a metallopeptidase is a functional receptor of the virus and is responsible for binding to S protein (Figure 1) and mediating

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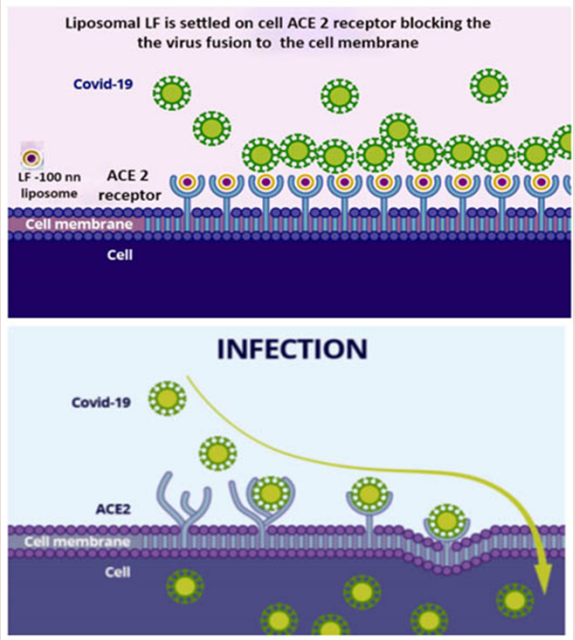

Figure 1: COVID-19 is being blocked by liposomal lactoferrin (up); virus affinity for ACE 2 receptor without interference by lactoferrin allow virus entrance inside the cell (down).

the virus entry into the target cells.[7] A segment with amino acids 318-510 of the protein S1 is the receptor binding domain for the ACE2 receptor.[7] ACE2 is highly expressed on human lung alveolar epithelial cells, enterocytes of the small intestine and the brush border of the proximal tubular cells of the kidney. The localization of ACE2 expression are consistent with the tissue tropism and pathogenesis of SARS-Co infection.[7] Some patients with COVID 19 infection, develop an intense host immune response against the virus. The innate immune response plays an important role in decreasing the viral infection. Many genes may be involved in the innate immune response, such as that encoding lactoferrin, S100A9 and lipocalin 2 which participate in SARS-CoV clearance.[8,9,11] It was observed that LF gene expression was upregulated and that LF was highly elevated (150 folds) in SARS patients in comparison with healthy volunteers. In our experience, CSGF was also upregulated but instead GMCSF was down regulated (unpublished data) after healthy volunteers ingested liposomal LF. It is believed that LF in this infection may works by stimulating NK cell activity and neutrophil aggregation and adhesion.[16,17] LF is a multifunctional glycoprotein present in several body fluids including milk, saliva, tears, semen, vaginal fluid, nasal and bronchial secretions, gastrointestinal fluids and urine mucosal secretions and is also present in the neutrophilic granules of leukocytes.[`6] LF has a strong antiviral activity against RNA and DNA virus including human immunodeficiency virus[18], sika virus[19, Chikungunya[19], hepatitis C[12], Sindbis viru[13], cytomegalovirus[20], herpes simplex virus[21], human papillomavirus[22], and rotaviru[23]. These viruses utilize common molecules such as heparan sulphate proteoglycans (HSPG) on the cell membrane to make easy the invasion into cells. These molecules provide the first anchoring sites on the cell surface and help the virus make primary contact with host cells [7]. LF may be able of preventing the internalization of some viruses after binding to HSPGs, which is present on most cells [7]. This property of LF confers protection to the host against viral infections. LF has an important protective role in the host immune defense against COVID 19 invasion.

MATERIALS AND METHODS

Seventy- five patients affected by COVID-19 showing the typical symptoms of the disease and testing positive for SARS-CoV-2 were selected for inclusion in the study. Patients were isolated and treated at home using remote systems, assessed daily for 10 days, and followed up for up to one month. Diagnosis was confirmed by SARS-CoV-2 IgM/IgG antibody rapid test done in whole blood (SARS-CoV-2 IgM/IgG antibody rapid test (Liming Bio, Jiangsu, P.R. China). LLF (Lactyferrin™ Forte drinkable, Sesderma Laboratories, Valencia, Spain) as well as a liposomal zinc (LZ) syrup (Zinc Defense syrup, Sesderma Laboratories, Valencia, Spain) were administered orally in 4 to 6 doses per day. LZ solution was administered at the dose of 10 mg/10 ml twice or three times a day. A control group of 12 patients also received only LLF. The LLF total daily dose ranged between 256 and 384 mg/day. All family members who had contact with the patients (256 persons) were also treated with half of this dose. Patients presenting with headache, dry cough and nasal congestions were also treated with LL-nasal drops and mouth spray (Lactyferrin nasal drops and Lactyferrin mouth spray) applied 4 times a day. LL in aerosol (SES Nanomist, Sesderma Laboratories; Figure 2) was also administered to all patients experiencing breathing difficulties. All the participants were assessed remotely by the medical team. Patients were monitored daily (at least twice a day) for 10 days and then after 30 days. For every patient and at every session symptoms were scored on a scale of 0 to 3, as follows: 0 absence of symptoms; 1 mild symptoms; 2 moderate symptoms; and 3 severe symptoms. Taste and smell were evaluated on a scale of 0 to 5, where 0 represented the absence of taste/smell (ageusia/anosmia) and 5 non-affected taste/smell.

RESULTS

For the group with the combination treatment (LLF + LZ), the median age of the patients was 42 years; 45% were female. We also included a separated group of four patients who underwent mechanical ventilation and were treated in the hospital. All were alive at the time of follow up. The most commonly reported symptoms were weakness/tiredness (94,44%), loss of smell (83,33%) and taste (88,89%), muscular pain (66,67%) dry cough (61,11%), headache (55,56%), diarrhea (44,4%), runny nose (33,33%), breathing difficulties (27,78%), nasal congestion (22,22%) and odynophagia (22,22%). Other symptoms include fever (38%), cramps (30%), insomnia (50%), night agitation (30%), nausea and intense stomach pain, flatulence, sore throat (28%), while one patient complained of abrupt and heavy hair loss (1,3%). No analytical data (radiography or computed tomography) were collected. Patients treated in the hospital were not included as to date we do not have a complete data set.

Day 0: Data was collected during the first remote contact with the participants. Treatment had noy yet begun (Table 1). The percentages of symptoms observed are shown in Table 2 ordered according to severity.

48 hours: Data was collected 48 hours after the first contact with the participants. Patients had already started treatment and some improvements could be observed (Table 3).

Figure 2: Liposomal lactoferrin nebulizer.

Table 1: Absence or presence of symptomatology from patients included in the study before receiving the treatment (day 0).

Symptomatology (%)	NO	YES
Dry cough	38,89	61,11
Productive cough	77,78	22,22
Respiratory distress	72,22	27,78
Muscular pain	33,33	66,67
Rhinorrhea	66,67	33,33
Nasal congestion	77,78	22,22
Taste	11,11	88,89
Smell	16,67	83,33
Odynophagia	77,78	22,22
Tiredness	5,56	94,44
Diarrhea	55,56	44,44
Headache	44,44	55,56

Table 2: Degree of involvement of symptoms from patients included in the study before receiving the treatment (day 0).

Symptomatology (%)	0	1	2	3	4	5
Dry cough	38,89	22,22	33,33	5,56	-	-
Productive cough	77,78	16,67	5,56	0	-	-
Respiratory distress	72,22	27,78	0	0	-	-
Muscular pain	33,33	16,67	27,78	22,22	-	-
Rhinorrhea	66,67	22,22	11,11	0	-	-
Nasal congestion	77,78	11,11	11,11	0	-	-
Taste	72,22	11,11	0	5,56	0	11,11
Smell	72,22	11,11	0	0	0	16,67
Odynophagia	77,78	22,22	0	0	-	-
Tiredness	5,56	11,11	50	33,33	-	-
Diarrhea	55,56	33,33	11,11	0	-	-
Headache	44,44	27,78	5,56	22,22	-	-

Table 3: Absence or presence of symptomatology from patients after 48 hours of treatment.

Symptomatology (%)	NO	YES
Dry cough	50	50
Productive cough	88,89	11,11
Respiratory distress	100	0
Muscular pain	55,56	44,44
Rhinorrhea	83,33	16,67
Nasal congestion	94,44	5,56
Taste	11,11	88,89
Smell	16,67	83,33
Odynophagia	88,89	11,11
Tiredness	33,33	66,67
Diarrhea	88,89	11,11
Headache	100	0

After the first 48 hours of treatment, headache symptoms disappeared in 100% of patients. The percentage of patients with a dry cough decreased from 61.11% to 50%. These patients reported that they were experiencing significant relief of these symptoms and associated with the application of the nasal drops and mouth spray. The percentage of patients experiencing muscle pain decreased from 66.67% to 44.44% while the percentage of patients experiencing tiredness/weakness decreased from 94.44% at the start of the study to 66.67% after 48 hours. For all the patients showing moderate to severe dyspnea LF nebulization was performed using a Nanomist nebulizer (SES Nanomist, Sesderma laboratories). No significant improvement in taste or smell was reported after 48 hours (Table 3). Improvements in taste and smell were slower compared with the rest of the symptoms. As shown In Table 4 (% severity scale for the symptoms) 72.22% of the patients who had a total loss of taste and smell (ageusia/anosmia) at day 0) (Table 2), presented a reduction at 48 hours (44,44% and 38,89% for taste and smell respectively).

Five day. All the participants continued to improve and continued the 10-day treatment schedule. At day 5th, the percentage of patients with a dry cough decreased from 61.11% at the start of the study to 38.89%. The percentage of patients experiencing muscle pain was reduced from 66.67% to 22.22% while the percentage of those reporting tiredness/weakness decreased from 94.44% to 27.78%. As was observed at 48 hours, 100% of the patients reported having no headache on day 5. In contrast to other symptoms, no significant improvement in taste and smell was reported (Table 5). The recovery of the smell and taste of patients was slower. However, a progressive improvement in symptoms was observed (Table 6; % grade of severity of symptoms) and from about 72.22% of patients with total loss of taste and smell (ageusia/anosmia) at day 0, the percentage was reduced to 38,85 at day 5.

Table 4: Degree of involvement of symptoms from patients after 48 hours of treatment.

Symptomatology (%)	0	1	2	3	4	5
Dry cough	50	33,33	16,67	0	-	-
Productive cough	88,89	11,11	0	0	-	-
Respiratory distress	100	0	0	0	-	-
Muscular pain	55,56	38,89	5,56	0	-	-
Rhinorrhea	83,33	16,67	0	0	-	-
Nasal congestion	94,44	0	5,56	0	-	-
Taste	44,44	22,22	11,11	11,11	0	11,11
Smell	38,89	22,22	22,22	0	0	16,67
Odynophagia	88,89	11,11	0	0	-	-
Tiredness	33,33	44,44	16,67	5,56	-	-
Diarrhea	88,89	5,56	5,56	0	-	-
Headache	100	0	0	0	-	-

Table 5: Absence or presence of symptomatology from patients after 5 days of treatment.

Symptomatology (%)	NO	YES
Dry cough	61,11	38,89
Productive cough	94,44	5,56
Respiratory distress	100	0
Muscular pain	77,78	22,22
Rhinorrhea	88,89	11,11
Nasal congestion	88,89	11,11
Taste	11,11	88,89
Smell	16,67	83,33
Odynophagia	88,89	11,11
Tiredness	72,22	27,78
Diarrhea	94,44	5,56
Headache	100	0

Table 6: Degree of involvement of symptoms from patients after 5 days of treatment.

Symptomatology (%)	0	1	2	3	4	5
Dry cough	61,11	38,89	0	0	-	-
Productive cough	94,44	5,56	0	0	-	-
Respiratory distress	100	0	0	0	-	-
Muscular pain	77,78	22,22	0	0	-	-
Rhinorrhea	88,89	11,11	0	0	-	-
Nasal congestion	88,89	11,11	0	0	-	-
Taste	38,89	11,11	16,67	11,11	11,11	11,11
Smell	38,89	5,56	22,22	11,11	5,56	16,67
Odynophagia	88,89	11,11	0	0	-	-
Tiredness	72,22	22,22	5,56	0	-	-
Diarrhea	94,44	5,56	0	0	-	-
Headache	100	0	0	0	-	-

POROGRESSION OF THE MAIN SYMPTOMS

The progression of the main symptoms of COVID-19 patients at day 0, 48 hours and 120 hours (day 5) is described below.

Dry cough (Figure 3): At the start of the study (day 0), 61.11% of the patients had a dry cough. After 48 and 120 hours of treatment, this percentage was reduced to 50% and 38.89% respectively. The treatment notably reduced the incidence of dry cough.

Breathing difficulty (Table 3): All the patients who reported difficulty breathing at the start of the study (day 0) showed some improvement at 48 hours.

Muscular pain (Figure 4): At the start of the study (day 0), 66.67% of the patients reported muscle pain. However, this percentage was reduced to 44.44% and 2.22% after 48 and 120 hours, respectively. The treatment significantly reduced the strong muscle pain associated with COVID-19.

Tiredness (Figure 5): At day 0, 94.44% of the patients reported feeling tired. After 48 and 120 hours of treatment, this percentage was reduced to 66.67% and 27.78% respectively.

Headache (Figure 6): At day 0, 55.56% of the patients presented with an intense headache. After 48 and 120 hours none of these patients reported having a headache.

Taste (Figure 7): At day 0, 72.22% of the patients had ageusia (total absence of taste), while 11.11% had no taste involvement. The rest of the patients had hypogeusia (partially reduced sense of taste). After 48 hours of treatment, the percentage of patients with ageusia decreased to 44.44% and then further decreased to 38.89% at 120 hours (day 5), implying a partial and progressive return of the sense of taste. At day 10, all the affected patients had completely recovered their sense of smell and taste. Most of the patients with ageusia of not being able to recognize the flavor of food (candy, sea food, chili).

Smell (Figure 8): At day 0, 72.22% of the patients had anosmia (total absence of smell), while 16.67% of patients showed no olfactory involvement. The rest of the patients had hyposmia (partial reduced sense of smell). After 48 hours, the percentage of patients with anosmia decreased to 38.89 and remained the same at 120 hours. The sense of smell had completely returned in 95% of the cases at day 10.

Results obtained in the control group taking LLL were very similar.

Analysis of these results indicated that oral treatment with LLF and LLF + LZ allows for fast recovery in 100% of patients within the first 4-5 days. The same treatment, but at a lower dose seems to exert a potential preventive effect against COVID-19 in healthy people directly related to the affected patients. Importantly, combination of the oral and topical

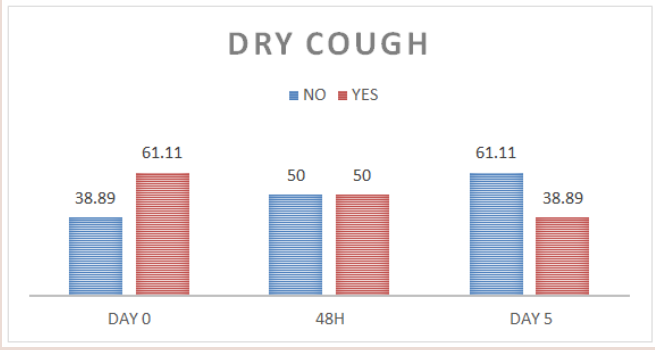

Figure 3: Absence or presence of dry cough from patients before treatmet (day 0) and after the treatment (48 hours and 5 days).

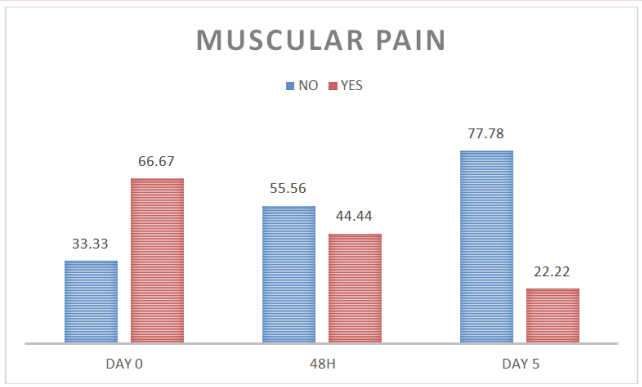

Figure 4: Absence or presence of muscular pain from patients before treatmet (day 0) and after the treatment (48 hours and 5 days).

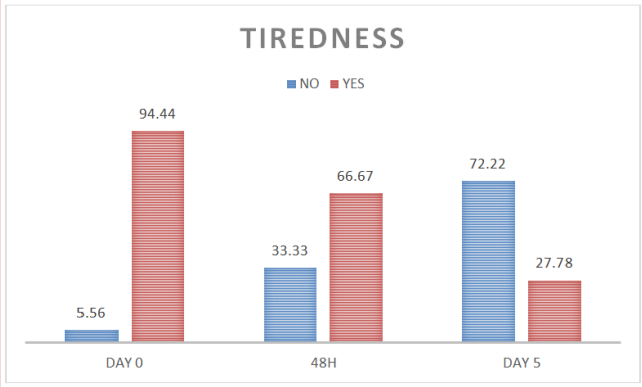

Figure 5: Absence or presence of tiredness from patients before treatmet (day 0) and after the treatment (48 hours and 5 days).

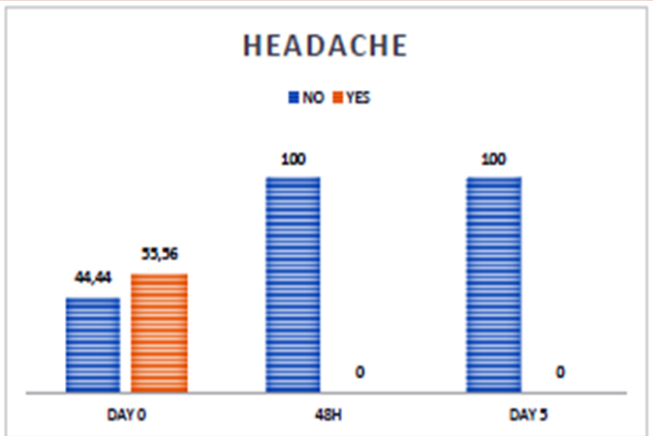

Figure 6: Absence or presence of headache from patients before treatment (day 0) and after the treatment (48 hours and 5 days).

treatments provided significant relief of the headache and dry cough. Some of these patients had sinus congestion. Dietary supplementation of LLF an LZ supported and enhanced the immune system response through their antioxidant, antibacterial, and antiviral properties.

DISCUSSION

The most frequent symptom in our patients was a very heavy sensation of tiredness or weakness (95%) followed by an alteration in the perception of smelling and taste (88%), cough (83%), muscular pain (67%), headache (56%) and diarrhea (44%). Most symptoms improved significantly during first five days in a percentage of improving of

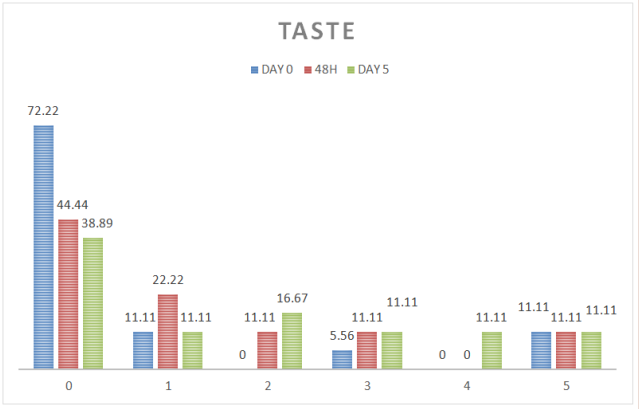

Figure 7: Absence or presence of taste from patients before treatmet (day 0) and after the treatment (48 hours and 5 days).

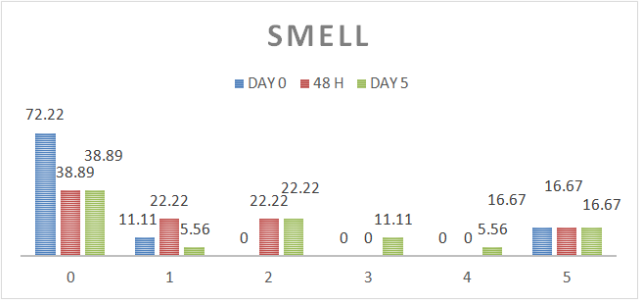

Figure 8: Absence or presence of smell from patients before treatmet (day 0) and after the treatment (48 hours and 5 days).

headache (100%), muscular pain (78%), taste (72%), smell (17%), weakness (72%) and dry cough (61%). At day all patients presented no symptoms and complete recovery was observed in all persons (100%). Olfactory and taste disfunction were the most predominant symptom in our cases and occurred very early reflecting the massive entrance of viral particles in the oral and nasopharyngeal mucosa. These alterations were described in the severe acute respiratory syndrome (SARS).[19,21,24] Patients were unable to recognize the taste of foods even sweet or spicy foods neither to smell (anosmia) fragrances or regular house cleansers (alcohol, kitchen detergents or chlorine bleachers). Angiotensin Converting Enzyme 2 receptor (ACE2) which are the main receptors for COVID-19 are highly expressed on the nose, mouth, particularly in the base of the tongue and throat sites for which the virus has great tissue tropism. Nose, mouth and throat topical care with nasal drops, mouth wash, throat sprays and even liposomal based toothpastes could be of help to reduce the viral load.[25] These alterations may be regarded as a transient olfactory and taste dysfunction and requires further follow up and investigation.[19,22,26] Patients having chemotherapy may improve taste and smell dysfunction by the intake of LF improving cancer patient's oral immunity. We found that the use treatment of LL nasal drops was very useful to relieve not only the respiratory symptoms but also the cough, the headache and the smell and taste dysfunction. A 28% of our patients presented with breathing difficulty. In these cases, we recommended the use of LF liposomal aerosol inhalation device 4 times a day with great success. The inhalation was well tolerated, and any side effect was reported. Inhalations through the LLF nebulizer was also recommended to two of the four patients admitted to hospitals. LF is a natural protein present in bronchial secretions and all the content in the liposome is biocompatible and biodegradable. Aerosol liposomal therapy have been employed for years with good results and a wide safety profile.[27-29] It has been employed with ribavirin in respiratory syncytial virus infection[30] and rhinovirus infections.[29] LF is a hydrophilic chemical that because of its encapsulation in PC liposomes behave as amphiphilic. LF reaches high enough concentrations within the lungs and provides a delayed release of LF in the target organ, the respiratory tract. The LF nanoliposome has a size of 100 nm but once it is nebulized through the airways it has a reduced size of 50 nm because on the hole of the device there is a filter that reduces the size of the nanosome. The liposome by itself may interact with the natural surfactant (phospholipid and proteins) of the target tissue (lungs) and PC may also exert a strong anti-inflammatory effect.[17,31,32] COVID- 19 lung infections may alter total pulmonary surfactant and changes its composition thus decreasing the availability of phospholipids which decreased pulmonary function as it happens in chronic obstructive pulmonary disease. Without surfactant, alveoli tend to collapse during normal tidal breathing, resulting in diminished lung compliance. Lactoferrin, is a globular protein belonging to the family of transferrins and showing great affinity for iron ions. Lactoferrin is a defense protein found in human milk, where it is present at relatively high concentration (1 g/L), especially in the calostral stage (up to 7 g/L). It is also present in other body fluids (tears, semen, vaginal fluid, saliva, bronchial secretions, etc.). It is synthesized by blood neutrophils and various types of cells including some acinar cells. The levels of endogenous lactoferrin increase during infection and inflammation. LF has antibacterial and antiviral properties, modulates the immune system and protects against intestinal inflammation.[33] Lactoferrin may influence leukocytes by increasing the activity of NK cells, neutrophils and macrophages. It increases the production of cytokines and nitric oxide and limits the growth of pathogens. Promotes maturation of immune cells, e.g. T and B lymphocytes. It can reduce the symptoms of allergies by blocking the release of histamine from intestinal mast cells. It can probably be helpful in the prevention and treatment of the effects of obesity and may promote reduction of visceral fat. It may help to improve the condition of the skin in ailments such as acne, skin inflammation and psoriasis. In the present study we use a liposomal lactoferrin (LLF), registered as a food supplement, Lactyferrin™, and we found this product to be safe and effective for treatment and prevention of COVID-19. 75 patients positive for COVID-19 infection were successfully treated. Most patients responded during the first 3 to 4 days of treatment with Lactyferrin™, although treatment was recommended for 10 days. Individuals in contact with symptomatic patients were also treated with half of the curative dose, resulting in disease prevention. The combination of liposomal LF and liposomal zinc is very effective and safe for treatment of this potentially fatal disease. The liposomal bovine LF-based nutritional food supplement, Lactyferrin™ Forte drinkable (Sesderma laboratories, Valencia, Spain) is registered in the European Union (Ireland), as well as by the United States Food and Drug Administration, as a food supplement. The European Food Safety Authority recognizes bovine LF as a dietary supplement with medicinal properties, and it is classified as a novel, safe ('Generally Recognized as Safe') food, with no contraindications. Total adult doses of non-encapsulated LF range between 1.4 and 3.4 g, and this supplement is frequently used to strengthen the immune system. Lactyferrin contains LF (32 mg/10 ml and vitamin C 12 mg/10 ml). Both substances are hydrophilic and have very limited gastric absorption. The liposome, with its closed phospholipid bilayer vesicular system, could encapsulate both hydrophilic (lactoferrin, vitamin C, zinc) and hydrophobic drugs (vitamin A). The liposomes we made are based on phosphatidyl choline (PC) which is a biocompatible and biodegradable chemical.[17,20,31,34] LF in its free form is degraded within the stomach by the action of hydrochloric acid and hydrolytic enzymes (proteases, pepsin); hence, the bioavailability of the free form is very limited. Therefore, LF and vitamin C are encapsulated in a 100 nm lipid bubble or nanoliposome, made from soybean phosphatidylcholine (PC) in Lactyferrin™. The nano-liposome protects LF from destruction by digestive secretions and allows the intact protein to travel through the duodenum and reach the general circulation, from where its bioavailability is very high.[17,31,34] LF encapsulated is in this way is

protected from pepsin and hydrolysis by proteases. It is also important to note that free LF is rapidly cleared from the circulation, limiting its therapeutic potential. Therefore, it is necessary to encapsulate it in liposomes to improve plasma stability. The PC used to make liposomes is a ubiquitous, naturally occurring phospholipid molecule, which is the major lipid in cell membranes and blood proteins. Further, PC serves as the main physiological source of choline, an essential nutrient and precursor to the neurotransmitter, acetylcholine. PC is also necessary to produce surfactants, which are critical for lung function and gastrointestinal health. The terms "phosphatidylcholine" and "lecithin" are sometimes used interchangeably; however, lecithin is a mixture of several lipids and phospholipids. PC is necessary for the composition and repair of cell membranes and vital for normal liver function. Research indicates that PC has a beneficial role in the prevention and treatment of various forms of liver disease and toxicity. PC protects liver cells from viral damage, reduces fibrosis, and prevents cell death from drugs, alcohol, and other chemical toxins.[5] The protective effects of LF range from anticancer, anti-inflammatory, and immune modulator properties, to antimicrobial, antifungal, and antiviral activities against many microorganisms.[18,35-37] This wide range of activities is made possible by mechanisms of action involving, not only the capacity of LF to bind iron, but also interactions of LF with molecular and cellular components of both hosts and pathogens. The antibacterial activity of LF is related to deprivation of environmental iron, which is essential for bacterial growth, while its antiviral activity[37-39] is associated with its role as a competitor of cell membrane receptors commonly used by viruses to enter cells. Specifically, LF is an ACE2 blocker, and prevents the binding of the virus spike protein S to the host cell, blocking the virus from fusing with the cell membrane.[8-12] Liposomal LF can also suppress viral replication after entry into the cell, as in the case of HIV.[18,37] Furthermore, some HIV-1 infected patients show decreased levels of plasma LF and in others, the lack of lactoferrin (and secretory IgA) found in the oral cavities of persons with HIV correlated strongly with the frequent infections in those areas.[38] Nanoliposomes also have beneficial effects related to their size and composition (PC); they are smaller size (100 nm) than the virus (150 nm), they can compete to reach target cells, where they settle before the virus. We have also demonstrated that specific doses of liposomal LF can prevent COVID-19. Lower doses do not prevent the infection (unpublished data). In addition, using lactoferrin, we have contributed to successful treatment of at least four intubated hospitalized patients, who were high-risk (high ferritin, IL-2, DD2 levels) and had virtually absent vital signs. LF significantly reduces the hyperimmune reaction observed in patients in a critical condition and suffering from an aggressive pro-inflammatory cytokine (IL-2 and 6) storm, normalizing or decreasing IL-6, TNF alpha, ferritin, and DD2 parameters, and protecting the lungs against acute respiratory distress. LF has immunomodulatory and anti-inflammatory properties[32,40], which are important in the pathophysiology of serious infections. Further, LF has a crucial immunomodulatory role in maintaining immune and physiological homeostasis and limiting tissue damage by modulating cytokines, chemokines, and cell surface receptors involved in signaling cascades.[41-45] The restorative and homeostatic roles of LF are notable in the context of the 'systemic inflammatory response', which describes the physiological response to serious insults such as sepsis.[23,41-45] The concept of a 'cytokine storm'[46], reflects the hyper-induction of inflammatory responses that result from uncontrolled immune activation[43], and these clearly respond to oral administration of LF.[43] LF is useful for treatment of the most severe cases of COVID- 19, because of its ability to modulate overreactive immune and inflammatory responses to viral infections, as we have observed in at least four patients. LLF might play a role in the disturbances of iron metabolism observed during inflammation and conditions with increased neutrophil turnover. In this event, a lot of released cytokines and/or inflammatory products such as interleukin 1, endotoxin), TNF-alpha and immune complexes may trigger the release of lactoferrin from the neutrophils. Lactoferrin will bind to its membrane receptor in monocytes/macrophages and trapped in this position it might prevent the transfer of iron from the macrophage to serum transferrin. Iron released from senescent phagocytosed red cells might in this way be captured by membrane-bound lactoferrin and transferred back to intracellular ferritin. We have been using liposomal LF to treat various medical conditions for the past 14 years. We also tested the neuroprotective effects of liposomal LF in a *Caenorhabditis elegans* model by evaluating both phenotypic and transcriptome responses.[33] The LF-based product protected against acute oxidative stress and extended the lifespan of *C. elegans* in a dose-dependent manner. Furthermore, paralysis of the transgenic *C. elegans* strain, CL4176, caused by Aβ1-42 aggregates, was clearly ameliorated by treatment with LF. Transcriptome analysis of treated nematodes indicated that it led to immune system stimulation, together with enhancement of processes involved in the oxidative stress response. The LF-based product also improved processes involved in protein homeostasis, cellular adhesion, and neurogenesis in the nematode. We conclude that LF provides protection against aging and neurodegeneration, modulating processes involved in the oxidative stress response, protein homeostasis, synaptic function, and xenobiotic metabolism. The liposomal LF-based product was also able to stimulate the immune system, as well as improving reproductive status and energy metabolism. Together, all these findings suggest that oral supplementation with liposomal LF could benefit the immune system and improve antioxidant capacity.[31] Many patients admitted to our local hospitals with COVID-19 are over the age of 70 and have very low zinc levels, which can contribute to the severity of the infection. Zinc is also hydrophilic and exhibits poor absorption through the gastrointestinal tract. Interestingly, the administration of nano encapsulated zinc might support the recovery of patients with COVID 19 infection. Zinc have also exhibited a potent antiviral effect. In polio virus experiments[30] zinc inhibited viral infection when incubated with cells after viral fusion, and the level of inhibition was correlated with the degree of zinc saturation.[30,33,47-49] Zinc supplements have previously been proposed for administration to patients with COVID-19.[49] Zinc may also influence the metalloproteases involved in the process of coronavirus fusion, by decreasing both cell entry and cell-cell fusion. LF can be used along or in combination with zinc and both supplements are non-toxic and can also be used as adjuvant treatments, alongside conventional antiviral drugs or hydroxychloroquine, as shown for treatment of hepatitis C virus (HCV)[24], where LF decreased the HCV RNA titer by contributing to the effectiveness of therapy with combined interferon and ribavirin. LF have a great potential for use as an adjunct treatment for patients with viral diseases. From our experience with COVID-19 home-isolated patients, and careful contact tracing, we conclude that liposomal LF can prevent and cure the infection in a dose dependent manner. This treatment is also indicated in patients with severe disease, as we observed in four patients who were critically ill. The doses we recommend for treatment and prevention of COVID-19 are provided in the addendum below. This mentioned treatment is completely free of side effects. Empirical treatment with hydroxychloroquine plus azithromycin[50-52] is being promoted by some institutions. However, clinical studies are not completed and hydroxychloroquine is not free of side effects and may induce a wide range of adverse effects. Cardiovascular, dermatologic, gastrointestinal, hematologic, hepatic, hypersensitivity, metabolic, musculoskeletal, nervous system, ocular, psychiatric and respiratory side effects have been described. In particular, cardiac rhythm alterations have been observed in a patient with systemic lupus erythematosus who developed syncopal episodes resulting from significant QT interval prolongation. This was corrected after discontinuation of the drug.[51] The administration of azithromycin is a paradox. Antibiotics don't have any effect on virus and the drug is administered because of its immune modulatory effect, but unfortunately azithromycin[52] shares with hydroxychloroquine the potential to induce QT-segment prolongation causing abnormal changes in the electrical activity of the heart that

may lead to a potentially fatal irregular heart rhythm.[52] Antibiotics disturb the intestinal microbiota which already has been destroyed by COVID-19 and probiotics administration is recommended. Elderly patients taking antibiotics may develop intestinal dysbiosis with changes in the gut microbiota rendering these patients prone to heart failure.[53] In our series, 94,44% of the patients developed diarrhea which LLF counteract before the 5th day. LLF may exert is anti-inflammatory effect both at gastrointestinal level balancing the local microbiota and reducing the intestinal damage induced by the virus. Lactoferrin increase the good micro flora—such as bifidus—and a decrease bad bacterium, such as *E. coli*, streptococcus, clostridium and others. Lactoferrin acts as anti-inflammatory agent promoting the "good" cytokines such as interleukin (IL)-4 and IL-10 and reducing the proinflammatory cytokines such as tumor necrosis factor-alpha, IL-6 and IL-1 beta, and downregulation of the nuclear factor-kappa.[44] The best of the treatment with LF is that is simple, safe and potentially effective preventative and treatment of COVID 19 infections, that may be useful for young adults, elderly persons, children and pregnant women. Use of LF encapsulated in a very tiny PC-liposomes is recommended for a maximum anti-inflammatory and immunomodulatory action and for protection of the LF-protein content from the deleterious effect of the enzymes and acids in the stomach. At the moment of closing the present paper, Chang *et al.* empirically proposed the use of nutritional supplements of LF and commented that LF show antiviral efficacy against a wide range of virus including SARS-CoV, a closely related corona virus to SARS-COV-2 (COVID-19) and also possesses significant immunomodulatory and anti-inflammatory properties which might be of relevance to the pathogenesis of severe COVID-19 cases. Our clinical trial confirmed their assumption and elucidate the potential doses for prevention and treatment of COVID-19 infection.[54-57]

ADDENDUM

Liposomal LF (Lactyferrin™) doses

Treatment dose: 64-96 mg (20-30 ml) every 6 h daily to cure COVID-19 (256-384 mg/d). Doses can be increased to 128 mg every 6 h (512 mg) if needed.

Preventive dose: 64 mg two to three times daily can prevent COVID-19 (128-192 mg/d).

Lactyferrin pregnancy and babies' syrup (glycerosome encapsulation, alcohol free)

- Pregnant women and infants under the age of two.

- Mothers: 64 mg (20 ml) twice a day (128 mg/d).

- Infants: 32 mg (10 ml) twice daily.

- Zinc Defense syrup: 10-30 mg/d (10-30 ml)

LF nasal drops (Lactyferrin): These nasal drops contain nano LF to quickly relieve acute sinusitis and the alterations in smell and taste experienced by many patients, while contributing to the management of dry cough. In acute cases, we recommend applying two drops to each nostril every 4-6 h.

CONFLICT OF INTEREST

GS, IK, AA, ED, MO & JMS all work for Sesderma laboratories.

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